PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number: WO 93/05812
A61K 39/21, C07K 7/08	A1	(43) International Publication Date: 1 April 1993 (01.04.93)

(21) International Application Number: PCT/US92/07714

(22) International Filing Date: 18 September 1992 (18.09.92)

(30) Priority data:
760,530 18 September 1991 (18.09.91) US

(71) Applicant: THE GOVERNMENT OF THE UNITED STATES OF AMERICA as represented by THE DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Box OTT, Bethesda, MD 20892 (US).

(72) Inventors: BERZOFSKY, Jay, A. ; 9321 Corsica Drive, Bethesda, MD 20814 (US). TAKAHASHI, Hidemi ; 3-49-11, Wada, Suginami-ku, Tokyo 166 (JP). GERMAIN, Ronald, N. ; 10404 Crossing Creek Road, Potomac, MD 20854 (US).

(74) Agents: SVENSSON, Leonard, R. et al.; Birch, Stewart, Kolasch & Birch, 301 North Washington Street, P.O. Box 747, Falls Church, VA 22046-3487 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).

Published
With international search report.

(54) Title: METHOD TO INDUCE CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR A BROAD ARRAY OF HIV-1 ISOLATES USING HYBRID SYNTHETIC PEPTIDES

(57) Abstract

The instant invention describes the synthesis of short peptides, corresponding to the amino acid residues of the V3 loop of the gp160 envelope glycoprotein of HIV-1 numbered 315 to 329 by Ratner (Ratner, L. et al., Nature 313, 277 (1985)) in the strain IIIB, wherein the residue corresponding to number 325 in HIV-1 IIIB is substituted by the homologous residue from another clinical isolate or strain. The invention further describes the use of said peptides in pharmaceutical compositions and an immunization protocol which elicits cytotoxic T cells reactive to a broad range of isolates of HIV-1.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SK	Slovak Republic
SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
UA	Ukraine
US	United States of America

METHOD TO INDUCE CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR A BROAD ARRAY OF HIV-1 ISOLATES USING HYBRID SYNTHETIC PEPTIDES

Field of the Invention

The invention is directed to a series of synthetic peptides useful as vaccines for the prophylaxis or immunotherapy of HIV-1 virus infection. The invention is further directed to pharmaceutical compositions and an immunization protocol utilizing the synthetic peptides to produce cytotoxic T-lymphocytes with cross-reactivity to a broad range of clinical isolates of HIV-1.

Background of the Invention

Scientific publications referred to in this application are hereby incorporated by reference.

The envelope glycoprotein gp160 has been used in numerous prototype vaccine preparations designed for prophylaxis against or immunotherapy of infection by HIV-1 or its close simian lentivirus relatives (Berman, P. W. et al., *Nature* **345**, 622 (1990); Zagury, D. et al., *Nature* **332**, 728 (1988).; Clerici, M., et al., *Eur. J. Immunol.* **21**, 1345 (1991); Redfield, R. R. et al., *N. Engl. J. Med.* **324**, 1677 (1991)). Studies in man and mouse have revealed a small region of this protein, called the V3 loop, between cysteine residues 303 and 338, that

evokes the major neutralizing antibodies to the virus (Palker, T.J., et al, *Proc. Natl. Acad. Sci. U. S. A.* **85**, 1932 (1988); Rusche, J.R., et al., *Proc. Natl. Acad. Sci. U. S. A.* **85**, 3198 (1988); Goudsmit, J. et al., *Proc. Natl. Acad. Sci. U. S. A.* **85**, 4478 (1988)) and stimulates both helper and cytotoxic T cell responses in both mice and humans (Takahashi, H. et al., *Proc. Natl. Acad. Sci. USA* **85**, 3105 (1988); Takahashi, H. et al., *J. Exp. Med.* **171**, 571 (1990); Clerici, M. et al., *Nature* **339**, 383 (1989); Clerici, M. et al., *J. Immunol.* **146**, 2214 (1991)). This same region is one of the most variable in sequence among different clonal isolates (Myers, G. et al., *Human retroviruses and AIDS 1989* (Los Alamos National Laboratory, New Mexico, 1989); LaRosa, G.J. et al., *Science* **249**, 932 (1990)), and this variation has been suggested to arise by selection of mutant virus as a result of the intense immune pressure directed against this region of the molecule (Albert, J. et al., *AIDS* **4**, 107 (1990); Nara, P.L. et al., *J. Virol.* **64**, 3779 (1990); Takahashi, H. et al., *Science* **246**, 118 (1989); Takahashi, H. et al., *J. Exp. Med.* **170**, 2023 (1989)). Thus, this segment of gp160 is both an attractive candidate for a major component of an AIDS vaccine because of its known antigenic properties, and a problem for the design of useful vaccines because of the extensive diversity in its structure already existing and likely to arise in the future.

Pircher et al. (Pircher, H. et al., *Nature* **346**, 629 (1990)) have directly demonstrated that the LCMV virus can escape cytotoxic T lymphocyte (CTL) immune

responses by accumulation of point mutations affecting T cell recognition even while preserving MHC molecule binding and display. Because CTL are likely to play a major role in effective immune responses against HIV-1, due to its capacity for direct intercellular transfer, we have examined the response of mice to this region of gp160. Our previous studies revealed the ability of a single residue change in the 315-329 immunodominant determinant, numbered according to the system of Ratner (Ratner, L. et al., *Nature* **313**, 277 (1985)), presented by the class I MHC molecule D^d to completely and reciprocally alter recognition by CTL directed against the MN and IIIB forms of this site (Takahashi, H. et al., *Science* **246**, 118 (1989)). This naturally occurring variation in T cell epitopes of gp160 might well be explained by the type of immune selection studied by Pircher et al. (Pircher, H. et al., *Nature* **346**, 629 (1990)), as this site is seen by human CTL specific for the HIV envelope (Clerici, M. et al., *J. Immunol.* **146**, 2214 (1991)) in addition to neutralizing antibodies (Palker, T.J., et al, *Proc. Natl. Acad. Sci. U. S. A.* **85**, 1932 (1988); Rusche, J.R., et al., *Proc. Natl. Acad. Sci. U. S. A.* **85**, 3198 (1988); Goudsmit, J. et al., *Proc. Natl. Acad. Sci. U. S. A.* **85**, 4478 (1988)). Because an effective anti-HIV vaccine strategy must anticipate to the greatest extent possible such potential changes in viral antigenicity, we have examined in detail the specificity of CTL recognition of numerous HIV-1 isolates and describe a method for immunization that generates broadly reactive CTL with an enhanced

capacity to respond to a wide array of variant sequences at this critical immunodominant site.

Summary of the Invention

It is one object of the invention to provide a peptide or group of peptides, useful for the prophylaxis or immunotherapy of HIV-1 infection, which elicits in the immunized subject cytotoxic T lymphocyte activity against a broad range of clinical isolates of HIV-1. It is a further object of the invention to provide for a pharmaceutical composition including at least one of such peptides and to provide for a method of immunization utilizing said pharmaceutical composition to elicit cytotoxic T lymphocyte response to a broad range of clinical isolates of HIV-1 in the immunized subject.

Brief Description of the Drawings

Figure 1 shows the effect of position 325 substitutions on CTL effector function. CTL-lines specific for either the IIIB (closed bar) or MN (open bar).

Figure 2 shows the relative sensitization potencies of substituted MN peptides. CTL line specific for IIIB (panel A) or MN (panel B) were co-cultured with ^{51}Cr-labeled BALB/c 3T3 fibroblast targets in the presence of the indicated concentrations of peptides at a 5 to 1 effector to target ratio.

Figure 3 shows restimulation of the IIIB-gp160 primed immune cells with substituted MN peptides. At the top of each panel, the designation before the slash indicates the recombinant vaccinia virus used

for immunization, and that after the slash the peptide used for restimulation in vitro.

Detailed Description of the Invention

Preferred embodiments of the invention are herein described by means of several examples. These examples are meant to be illustrative, rather than limiting in scope. It is to be understood that slight changes in techniques or materials would be readily obvious to one skilled in the art and such are to be considered within the scope of the present invention.

Example 1

Demonstration of the specificity of induction of cytotoxicity by gp160 and restimulation with homologous synthetic polypeptides of sequences identical to the V3 region amino acids 315 through 329 of natural isolates of HIV-1.

A. Peptide synthesis.

A series of peptide analogues of 18MN are synthesized by solid phase peptide synthesis (J. M. Stewart, J. D. Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Company, Rockford, Illinois, 1984), and purified by gel filtration and HPLC.

B. Immunization of mice and T-lymphocyte cytotoxicity assays.

Mice are immunized i.v. with 10^7PFU of recombinant vaccinia viruses, vSC25, vMN or vRF. vSC8 (recombinant vaccinia virus containing the bacterial lacZ gene), vSC-25, vMN, and vRF (recombinant vaccinia viruses expressing the HIV env

glycoprotein gp160 of the HIV IIIB, MN, RF isolates, respectively, without other HIV structural or regulatory proteins) have been previously described (Takahashi, H. et al., *Science* **246**, 118 (1989); Chakrabarti, S. et al., *Nature* **320**, 535 (1986)). Four to 8 weeks later, immune spleen cells (5 $x10^6$/ml in 24-well culture plates in complete T-cell medium (Takahashi, H. et al., *J. Exp. Med.* **170**, 2023 (1989)) are restimulated for 6 days *in vitro* with either 0.3μM of peptide 18IIIB (RIQRGPGRAFVTIGK) (Seq. I.D. No. 1), representing residues 315 to 329 of the HIV-1 strain IIIB gp160 envelope protein in the numbering system of Ratner et al. (Ratner, L. et al., *Nature*, **313**, 277 (1985)), 1μM of 18MN peptide (RIHIGPGRAFYTTKN) (Seq. I.D. No. 2), or 1μM 18RF peptide (SITKGPGRVIYATGQ) (Seq. I.D. No. 3) plus 10% Rat Con-A supernatant-containing medium (Rat T-cell Monoclone) (Collaborative Research, Inc., Bedford, MA). After culture for 6 days, cytolytic activity of the restimulated cells is measured as previously described using a 6 hr assay with various ^{51}Cr-labelled targets. For testing the peptide specificity of CTL, effectors and ^{51}Cr-labelled targets are mixed with various concentrations of peptide at the beginning of the assay (Takahashi, H. et al., *Proc. Natl. Acad. Sci. USA* **85**, 3105 (1988). The variant peptides are synthesized as previously described (Takahashi, H. et al., *Science* **246**, 118 (1989); Houghten, R.A. *Proc. Natl. Acad. Sci. USA.* **82**, 5131 (1985)). The percent specific ^{51}Cr release is calculated as 100x [(experimental release - spontaneous release)/ (maximum release - spontaneous release)]. Maximum release is determined from

supernatants of cells that are lysed by addition of 5% Triton-X 100. Spontaneous release is determined from target cells incubated without added effector cells.

We could elicit from BALB/c (H-2^d) mice CTL specific for the peptide SITKGPGRVIYATGQ (Seq. I.D. No.3) (18RF) the sequence of gp160 from the HIV-1 RF isolate which corresponds to the 315-329 region of gp160 from the IIIB isolate. These CTL did not crossreactively kill targets pulsed with homologous peptides from HIV-1IIIB or HIV-1MN (18IIIB or 18MN, respectively). Because we had already obtained HIV IIIB and MN envelope-specific CTL lines from BALB/c mice, both restricted by the same D^d class I molecule (Takahashi, H. et al., *Proc. Natl. Acad. Sci. USA* **85**, 3105 (1988); Takahashi, H. et al., *Science* **246**, 118 (1989)), we had three non-crossreactive, type-specific CTL lines that could kill targets infected with the appropriate gp160-expressing recombinant vaccinia virus as well as targets pulsed with the appropriate peptide. Taking advantage of these CTL lines and a series of synthetic peptides corresponding to the homologous portion of the 14 different HIV isolates shown in Table 1, we analyzed the crossreactivity of each CTL line for each peptide presented by H-2^d cells. The results are summarized in Table 1.

Table 1. Crossreactive CTL activity for the homologous portion of the gp160 immunodominant site from different HIV isolates.

HIV isolates	Sequence 315 325 329	Specific lysis (%)* at various peptide concentration: IIIB-specific CTL 10μM	IIIB-specific CTL 1μM	MN-specific CTL 10μM	MN-specific CTL 1μM	RF-specific CTL 10μM	RF-specific CTL 1μM	
IIIB	RIQRGPGRAF V TIGK	42.3	53.9	-0.5	-1.1	1.8	3.0	(SEQ. ID 1)
MN	RIHIGPGRAF Y TTKN	0.3	-2.3	43.3	50.3	1.5	1.7	(SEQ. ID 2)
RF	SITKGPGRVI Y ATGQ	0.3	-1.2	-0.9	-1.2	38.5	40.4	(SEQ. ID 3)
SC	SIHIGPGRAF Y ATGD	0.2	-1.6	46.2	42.2	3.0	-0.6	(SEQ. ID 4)
WMJ-2	SLSIGPGRAF R TREI	0.7	-1.2	4.2	-1.0	1.8	-0.1	(SEQ. ID 5)
Z321	SISIGPGRAF F ATTD	-0.3	-1.2	30.3	18.0	2.5	0.6	(SEQ. ID 6)
SF2	SIYIGPGRAF H TTGR	0.3	-0.4	25.9	13.8	1.6	0.4	(SEQ. ID 7)
NY5	GIAIGPGRTL Y AREK	-0.4	-0.7	10.2	1.0	1.4	-0.2	(SEQ. ID 8)
CDC4	RVTLGPGRVW Y TTGE	0.0	-2.0	1.0	-1.5	2.6	-0.2	(SEQ. ID 9)
Z3	SIRIGPGKVF T AKGG	0.5	-2.3	-1.7	-1.2	1.5	4.0	(SEQ. ID 10)
MAL	GIHFGPGQAL Y TTGI	0.2	-2.3	-1.4	-2.4	2.9	1.9	(SEQ. ID 11)
Z6	STPIGLGQAL Y TTRG	-0.6	-1.7	-2.1	-2.7	-0.7	-0.9	(SEQ. ID 12)
JY1	STPIGLGQAL Y TTRI	0.3	-2.3	1.2	-1.6	0.1	1.7	(SEQ. ID 13)
ELI	RTPTGLGQSL Y TTRS	0.4	-0.6	-0.5	-2.5	2.1	0.9	(SEQ. ID 14)

*Effector to target ratio is 10:1.

Neither IIIB-specific CTL nor RF-specific CTL crossreactively lyse targets incubated with peptides derived from other HIV isolates. However, MN-specific CTL do crossreactively kill targets incubated with the SC, Z321, SF2, and, weakly, NY5 derived peptides. Thus, significant crosskilling is observed with peptide sequences related to the prevalent MN type.

Example 2

Characterization of the specificity of cytotoxic T lymphocytes using gp160 from a natural HIV-1 isolate and restimulation with chimeric synthetic peptides.

Previous studies, have demonstrated that the amino acid at position 325 plays a critical role in the specificity of CTL responses to 18IIIB and 18MN (Takahashi, H. et al., *Science* **246**, 118 (1989); Takahashi, H. et al., *J. Exp. Med.* **170**, 2023 (1989)). This is consistent with the present observation that MN-specific CTL can strongly recognize targets sensitized with SC, Z321 and SF2 derived peptides, but not those incubated with WMJ-2 or IIIB peptides. These peptides share a common structure of -(I)-GPGRAF-X-(T)- , where X is a variable amino acid at position 325 and the residue present here determines target sensitivity to lysis by a given CTL population. To more systematically examine the effect of changes at this site on the lytic activity of the 18IIIB and 18MN CTL lines, we synthesized a series of substituted peptides each with a single amino acid substitution at position 325 in 18MN (J. M. Stewart, J. D. Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Company,

Rockford, Illinois, 1984). Table II describes the series of peptides synthesized in the experiments of the present example.

Table 2. A series of 18MN peptides with a single substitution at position 325.

Sequence	315										325				329		
18MN	R	I	H	I	G	P	G	R	A	F	Y	T	T	K	N		
18MN(Y-V)	R	I	H	I	G	P	G	R	A	F	V	T	T	K	N	(SEQ. ID 15)	
18MN(Y-I)	R	I	H	I	G	P	G	R	A	F	I	T	T	K	N	(SEQ. ID 16)	
18MN(Y-P)	R	I	H	I	G	P	G	R	A	F	P	T	T	K	N	(SEQ. ID 17)	
18MN(Y-L)	R	I	H	I	G	P	G	R	A	F	L	T	T	K	N	(SEQ. ID 18)	
18MN(Y-W)	R	I	H	I	G	P	G	R	A	F	W	T	T	K	N	(SEQ. ID 19)	
18MN(Y-F)	R	I	H	I	G	P	G	R	A	F	F	T	T	K	N	(SEQ. ID 20)	
18MN(Y-S)	R	I	H	I	G	P	G	R	A	F	S	T	T	K	N	(SEQ. ID 21)	
18MN(Y-E)	R	I	H	I	G	P	G	R	A	F	E	T	T	K	N	(SEQ. ID 22)	
18MN(Y-R)	R	I	H	I	G	P	G	R	A	F	R	T	T	K	N	(SEQ. ID 23)	
18MN(Y-H)	R	I	H	I	G	P	G	R	A	F	H	T	T	K	N	(SEQ. ID 24)	
18MN(Y-K)	R	I	H	I	G	P	G	R	A	F	K	T	T	K	N	(SEQ. ID 25)	
18MN(Y-Q)	R	I	H	I	G	P	G	R	A	F	Q	T	T	K	N	(SEQ. ID 26)	

A. Effect of position 325 substitutions on CTL effector function.

CTL-lines specific for either the IIIB (closed bar) or MN (open bar) HIV isolate gp160 are co-cultured during the CTL assay with ^{51}Cr-labeled BALB/c 3T3 fibroblast targets in the presence of a series of substituted MN peptides at 10μM. Effector to target ratio is 5 to 1.

As shown in Figure 1, the IIIB-specific CTL line can strongly lyse targets sensitized with peptide 18MN(Y-V) consisting of the 18MN sequence with 325 Y replaced by V, as we have already reported (Takahashi, H. et al., *Science* **246**, 118 (1989)). These CTL can also recognize the substituted peptides 18MN(Y-I) and 18MN(Y-L) in which 325(Y) is replaced by either I or L, respectively. The MN-specific CTL line can strongly lyse targets sensitized with 18MN(Y-F), 18MN(Y-H), or 18MN(Y-P) and moderately lyse targets with 18MN(Y-W), but fails to lyse cells treated with 18MN(Y-R). These results are compatible with the data in Table 1 that MN-specific CTL can crossreactively kill targets sensitized with Z321 (325(F)) or with SF2 (325(H)), but do not show crossreactivity for targets pulsed with 18WMJ-2 (325(R)). These results confirm and extend the conclusion that the amino acid at position 325 plays a major role in defining the specificity of CTL responses of H-2^d mice to the gp160 315-329 region (Takahashi, H. et al., *Science* **246**, 118 (1989); Takahashi, H. et al., *J. Exp. Med.* **170**, 2023 (1989)). They also suggest a chemical basis for cross-reactive CTL function, in that IIIB-specific

CTL tend to see an aliphatic amino acid at 325 like V, I, or L, whereas MN-specific CTL tend to see an aromatic amino acid like Y, F, H, or W or a ring structure such as P.

B. Comparison of the potency of the crossreactive peptides for the IIIB-specific CTL line.

CTL line specific for IIIB (panel A) or MN (panel B) are co-cultured with ^{51}Cr-labeled BALB/c 3T3 fibroblast targets in the presence of the indicated concentrations of peptides at a 5 to 1 effector to target ratio.

Either peptide concentration at the same effector to target ratio (Fig. 2) or effector to target ratio at a constant concentration of peptide is titrated. The results clearly demonstrate that the potency of 18MN(Y-V) is within ten-fold of that of the original peptide 18IIIB, whereas both 18MN(Y-I) and 18MN(Y-L) are 10 to 100 times less active (Figure 2A). Based on earlier evidence that residue 325 may interact with the T-cell receptor (Takahashi, H. et al., *Science* **246**, 118 (1989); Takahashi, H. et al., *J. Exp. Med.* **170**, 2023 (1989)), these results suggest that the IIIB-specific CTL T-cell receptor can distinguish fine differences among the three aliphatic amino acids (V, I, L). We also quantitated the activity of the 18MN, 18MN(Y-F), 18MN(Y-H), 18MN(Y-P), 18MN(Y-W) peptides using the MN-specific CTL line. As Figure 2B shows, the 18MN(Y-F) substitution reduce the ability to sensitize targets approximately 10-fold compared to 18MN, the 18MN(Y-H) and 18MN(Y-P) substitutions reduce the potency nearly 30-fold, and

the 18MN(Y-W) substitution reduce the potency 100-fold. Again, these comparisons indicate that MN-specific CTL T-cell receptors can distinguish the differences among five aromatic or cyclic amino acids (Y, F, H, P, W). Note that for both CTL lines, it is the bulkiest substitution in the relevant chemical category of amino acid that has the least activity, isoleucine in the case of the aliphatic category seen by the IIIB-specific line and tryptophan in the case of the aromatic category seen by the MN-specific line. This observation suggests that the T cell receptors of these CTL have relatively hydrophobic pockets, one set that distinguishes aliphatic from aromatic and the other vice versa, but each of which is too constrained to accept the bulkiest of the side chains in the corresponding category.

Example 3

Restimulation of the IIIB-gp160 primed immune cells with substituted MN peptides generates broader CTL specificities.

Highly isolate-specific CTL immunity upon vaccination is unlikely to provide appropriate protection against the range of HIV-1 variants present in the population. To examine whether a broader range of effector CTL specificities are induced by varying the epitopic structure of the antigen during priming and boosting, immune spleen cells from mice primed with recombinant vaccinia virus expressing IIIB gp160 are restimulated with either 18IIIB or several different 18MN-like peptides substituted at position 325. Immune spleen

cells from mice primed with recombinant vaccinia virus vSC25 expressing the IIIB-gp160 gene are restimulated 6 days with either 18IIIB or 18MN peptides (1 μM) substituted at position 325, plus IL-2. The resulting CTL are assayed on targets incubated with the indicated peptides.

The pattern of CTL crossreactivity induced by restimulation with the original 18IIIB appears the same as that of the IIIB-specific CTL line (Fig. 3A). However, we find that we can generate CTL populations with significantly broader specificity when the IIIB-gp160 primed spleen cells are restimulated with 18MN variant peptides containing aliphatic substitutions such as 18MN(Y-V), 18MN(Y-I), or 18MN(Y-L) (Figure 3B-D). Such CTL crossreactively lyse targets sensitized not only with the aliphatic substituted peptides themselves, but also targets exposed to 18MN(Y-F), 18MN(Y-R), 18MN(Y-K), and, more weakly, to 18MN, 18MN(Y-W), 18MN(Y-Q). Despite the cross-reactive killing by such effectors of targets sensitized with 18MN and 18MN-related peptides with aromatic or basic residues at 325, restimulation of IIIB-gp160 primed spleen cells with 18MN itself (325 Y) (Fig. 3E) or 18MN substituted peptides containing such aromatic or basic substitutions (Fig. 3F-H) does not induce any crossreactivity or indeed much specific CTL activity. The increased breadth of crossreactivity elicited by the procedure of priming with gp160 IIIB-expressing vaccinia virus and boosting with 18MN (Y-V) peptide is also apparent when the CTL are tested on natural HIV variant sequences. The CTL elicited by this procedure now lyse cells incubated

with peptides corresponding to isolates RF, MN, SF2, and WMJ-2 (26%, 28%, 12%, and 7% specific lysis, respectively), whereas CTL raised only against the IIIB isolate do not (< 1% lysis, see Table 1). They also lyse targets infected with recombinant vaccinia viruses vIIIB (vSC25) and vMN expressing the HIV-1IIIB and MN gp160 proteins endogenously, whereas CTL elicited by restimulation with the IIIB peptide only lysed targets infected with vIIIB.

These results show that enhanced cross-reactivity to a broad range of HIV-1 clinical isolates is attained by an immunization protocol which comprises a first immunization with a source of HIV-1 gp160 glycoprotein, followed by a second immunization with a synthetic chimeric peptide designed according to this invention. The chimeric polypeptide specifically consists of amino acid residues corresponding to residues 315-329 of the gp160 glycoprotein of HIV-1 strain IIIB from a first isolate or strain of HIV-1, except that the amino acid corresponding to residue 325 of IIIB is substituted with the homologous amino acid from a second isolate or strain. For example, in preferred embodiments of the invention, the chimeric polypeptide comprises the amino acids of the region homologous to 315-329 of strain IIIB that are obtained from strain MN, except that the tyrosine (Y) at the position homologous to 325 is substituted with valine (V), leucine (L) or isoleucine (I), designated herein as 18MN(Y-V), 18MN(Y-L) and 18MN(Y-I), respectively. This substitution is made because V is the amino acid at position 325 in HIV-1, strain IIIB, while L and I are structurally

similar to V in that all three are aliphatic amino acids.

The surprising result found according to the present invention is that the substitution of the position 325 amino acid from a second strain elicits cytotoxic T lymphocytes of increased cross-reactivity not just to that second strain, but to other strains as well. Thus a second presentation to the cellular immune system of the chimeric polypeptide according to the invention unexpectedly results in the production of cytotoxic T cells with an enhanced, broadened cross-reactivity to a broad range of HIV-1 isolates.

Example 4

Administration of recombinant vaccinia expressing gp160 and hybrid peptides as a vaccine against HIV-1

The aim of the research of a large number of biomedical researchers is the production of a vaccine which would produce protection to humans from infection by HIV-1 or therapeutic benefit in AIDS treatment. The instant invention provides peptides that may prove useful as candidates for such vaccines. A pharmaceutical composition including a vaccine in accordance with the present invention comprises an effective antigenic or therapeutic amount of at least one of the hybrid peptides and a pharmaceutically acceptable carrier such as physiological saline, non-toxic, sterile buffer and the like. Of course, additives such as preservatives, sterilants, adjuvants and the like, well known to one of ordinary skill in the art, could also be included in the pharmaceutical

composition to maintain or increase the efficacy of the preparation.

It is proposed that peptides of the instant invention can be administered as part of a vaccination protocol in a fashion similar to that for the administration to primates of a synthetic peptide vaccine against hepatitus B as described by Itoh (Itoh, Y. et al., *Proc. Natl. Acad. Sci. USA* 83:9174-9178 (1986)). An alternative method for the preparation of vaccines involves the use of Protein A coated microbeads that bind immune complexes of an antibody and the immunizing antigen on their outer surface (Platt, et al., U.S. patent number 4,493,825).

The administration of vaccinia virus as a vaccine is well-established art (Flexner, C. and Moss, B. in "New Generation Vaccines", pp. 189-206; G.C. Woodrow and M.M. Levine, eds. copyright 1990 by Marcel Dekker, New York, NY). In the present invention, the recombinant vaccinia virus portion of the immunization protocol is performed by such established techniques.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Berzofsky, Jay A.
Takahashi, Hidemi
Germain, Ronald N.

(ii) TITLE OF INVENTION: METHOD TO INDUCE CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR A BROAD ARRAY OF HIV-1 ISOLATES USING HYBRID SYNTHETIC PEPTIDES

(iii) NUMBER OF SEQUENCES: 26

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Birch, Stewart, Kolash & Birch
(B) STREET: 301 N. Washington
(C) CITY: Falls Church
(D) STATE: Virginia
(E) COUNTRY: USA
(F) ZIP: 22046-0747

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Svensson, Leonard R.
(B) REGISTRATION NUMBER: 30,330
(C) REFERENCE/DOCKET NUMBER: 1173-354p

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 703-241-1300
(B) TELEFAX: 703-241-2848
(C) TELEX: 248345

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HIV-1
(C) INDIVIDUAL ISOLATE: IIIB

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= peptide
/note= "synthetic peptide, sequence = residues 315 to 329 of HIV-1, isolate IIIB, gp160 envelope glycoprotein."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HIV-1
(C) INDIVIDUAL ISOLATE: MN

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= peptide
/note= "synthetic peptide, sequence = amino acids 315 - 329 of HIV-1, isolate MN, gp160 envelope glycoprotein"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 11
(D) OTHER INFORMATION: /label= substitution
/note= "substitution of V,I or L for Y at this position produces a "hybrid" peptide that elicits CTL specific for a broad range of HIV-1 isolates

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Arg Ile His Ile Gly Pro Gly Arg Ala Phe Trp Thr Thr Lys Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HIV-1

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= peptide
/note= "peptide 18MN(Y-F); synthetic, chimeric peptide; sequence = region of HIV-1 strain MN gp160 envelope glycoprotein that is homologous to residues 315-329 of strain IIIB, except that 325(Y) is substituted by (F)."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Arg Ile His Ile Gly Pro Gly Arg Ala Phe Phe Thr Thr Lys Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HIV-1

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= peptide
/note= "peptide 18MN(Y-S); synthetic, chimeric peptide; sequence = region of HIV-1 strain MN gp160 envelope glycoprotein that is homologous to residues 315-329 of strain IIIB, except that 325(Y) is substituted by (S)."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Arg Ile His Ile Gly Pro Gly Arg Ala Phe Ser Thr Thr Lys Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HIV-1

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= peptide
/note= "peptide 18MN(Y-E); synthetic, chimeric peptide; sequence = region of HIV-1 strain MN gp160 envelope glycoprotein that is homologous to residues 315-329 of strain IIIB, except that 325(Y) is substituted by (E)."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Arg Ile His Ile Gly Pro Gly Arg Ala Phe Glu Thr Thr Lys Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HIV-1

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= peptide
/note= "peptide 18MN(Y-R); synthetic, chimeric peptide; sequence = region of HIV-1 strain MN gp160 envelope glycoprotein that is homologous to residues 315-329 of strain IIIB, except that 325(Y) is substituted by (R)."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Arg Ile His Ile Gly Pro Gly Arg Ala Phe Arg Thr Thr Lys Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HIV-1

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= peptide
/note= "peptide 18MN(Y-H); synthetic, chimeric peptide; sequence = region of HIV-1 strain MN gp160 envelope glycoprotein that is homologous to residues 315-329 of strain IIIB, except that 325(Y) is substituted by (H)."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Arg Ile His Ile Gly Pro Gly Arg Ala Phe His Thr Thr Lys Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HIV-1

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= peptide
/note= "peptide 18MN(Y-K); synthetic, chimeric peptide; sequence = region of HIV-1 strain MN gp160 envelope glycoprotein that is homologous to residues 315-329 of strain IIIB, except that 325(Y) is substituted by (K)."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Arg Ile His Ile Gly Pro Gly Arg Ala Phe Lys Thr Thr Lys Asn
1 5 10 15

CLAIMS:

Claims

1. A synthetic, chimeric polypeptide comprised of amino acid residues from a first clinical isolate or strain corresponding to residues 315 to 329 of the gp160 envelope protein of HIV-1 strain IIIB, except that the residue homologous to that at position 325 of HIV-1 IIIB is substituted by the amino acid found at that position from a second clinical isolate or strain having a non-identical amino acid at that position, with the proviso that said polypeptide is not 18MN(Y-V).

2. A synthetic, chimeric polypeptide comprised of amino acid residues from a first clinical isolate or strain corresponding to residues 315 to 329 of the gp160 envelope protein of HIV-1 strain IIIB, except that the residue homologous to that at position 325 of HIV-1 IIIB is substituted by the amino acid found at that position from a second clinical isolate or strain having a non-identical amino acid at that position, wherein said isolate is an isolate selected from the group consisting of those isolates described in Table I, with the proviso that said polypeptide is not 18MN(Y-V).

3. A synthetic, chimeric polypeptide according to claim 1, wherein said first and second strains are selected from the group consisting of IIIB, MN and RF.

4. A synthetic polypeptide, according to claim 3, wherein said polypeptide has the amino acid sequence RIHIGPGRAFXTTKN, where X is a member selected from the group consisting of isoleucine, leucine, proline, tryptophan, phenylalanine, serine, glutamic acid, arginine, histidine, lysine and glutamine.

5. A synthetic polypeptide, according to claim 3, wherein said polypeptide has the amino acid sequence RIHIGPGRAFXTTKN, where X is a member selected from the group consisting of isoleucine and leucine.

6. A method of immunization for the induction of cytotoxic T-lymphocyte activity which comprises:

(i) a first immunization with a source of HIV-1 gp160 envelope glycoprotein,

(ii) at least a second immunization with at least one synthetic chimeric polypeptide comprised of amino acid residues from a first clinical isolate or strain corresponding to residues 315 to 329 of the gp160 envelope protein of HIV-1 strain IIIB, except that the residue homologous to that at position 325 of HIV-1 IIIB is substituted by the amino acid found at that position from a second clinical

isolate or strain having a non-identical amino acid at that position.

7. A method of immunization for the induction of cytotoxic T-lymphocyte activity, as described in claim 6, wherein at least one of the synthetic, chimeric polypeptides is of the sequence RIHIGPGRAFXTTKN, wherein X is an amino acid chosen from the group consisting of the members valine, leucine and isoleucine.

8. A method of immunization for the induction of cytotoxic T-lymphocyte activity, as described in claim 6, wherein the source of HIV-1 gp160 envelope glycoprotein is a recombinant vaccinia virus expressing said glycoprotein.

9. A method of immunization for the induction of cytotoxic T-lymphocyte activity, as described in claim 7, wherein the source of HIV-1 gp160 envelope glycoprotein is a recombinant vaccinia virus expressing said glycoprotein.

10. A method of immunization for the induction of cytotoxic T-lymphocyte activity, as described in claim 6, wherein the source of HIV-1 gp160 envelope glycoprotein is the recombinant vaccinia virus vSC25 or vMN or both.

11. A method of immunization for the induction of cytotoxic T-lymphocyte activity, as described in claim 7, wherein the source of HIV-1 gp160 envelope

glycoprotein is the recombinant vaccinia virus vSC25 or vMN or both.

12. A pharmaceutical composition for prophylaxis against or therapeutic treatment of HIV-1 infection comprising:

(i) a source of HIV-1 gp160 envelope glycoprotein,

(ii) at least one synthetic chimeric polypeptide comprised of amino acid residues from a first clinical isolate or strain corresponding to residues 315 to 329 of the gp160 envelope protein of HIV-1 strain IIIB, except that the residue homologous to that at position 325 of HIV-1 IIIB is substituted by the amino acid found at that position from a second clinical isolate or strain having a non-identical amino acid at that position, and

(iii) a pharmaceutically acceptable carrier, diluent or solvent.

13. A pharmaceutical composition for prophylaxis against or therapeutic treatment of HIV-1 infection comprising:

(i) at least one synthetic chimeric polypeptide comprised of amino acid residues from a first clinical isolate or strain corresponding to residues 315 to 329 of the gp160 envelope protein of HIV-1 strain IIIB, except that the residue homologous to that at position 325 of HIV-1 IIIB is substituted by the amino acid found at that position from a

second clinical isolate or strain having a non-identical amino acid at that position, and

(ii) a pharmaceutically acceptable carrier, diluent or solvent.

14. A pharmaceutical composition according to claim 12, wherein the source of HIV-1 gp160 envelope glycoprotein is a recombinant vaccinia virus.

15. A pharmaceutical composition according to claim 12, wherein the source of HIV-1 gp160 envelope glycoprotein is the recombinant vaccinia virus vSC25 or vMN or both.

16. A pharmaceutical composition according to claim 12, wherein said synthetic chimeric polypeptide is selected from the group consisting of the chimeric 18MN peptides described in Table 2.

17. A pharmaceutical composition according to claim 13, wherein said synthetic chimeric polypeptide is selected from the group consisting of the chimeric 18MN peptides described in Table 2.

18. A pharmaceutical composition according to claim 12, wherein the chimeric polypeptide has an amino acid sequence comprised of the sequence RIHIGPGRAFXTTKN, wherein X is an amino acid chosen from the group consisting of the members valine, leucine and isoleucine.

19. A pharmaceutical composition according to claim 13, wherein the chimeric polypeptide has an amino acid sequence comprised of the sequence RIHIGPGRAFXTTKN, wherein X is an amino acid chosen from the group consisting of the members valine, leucine and isoleucine.

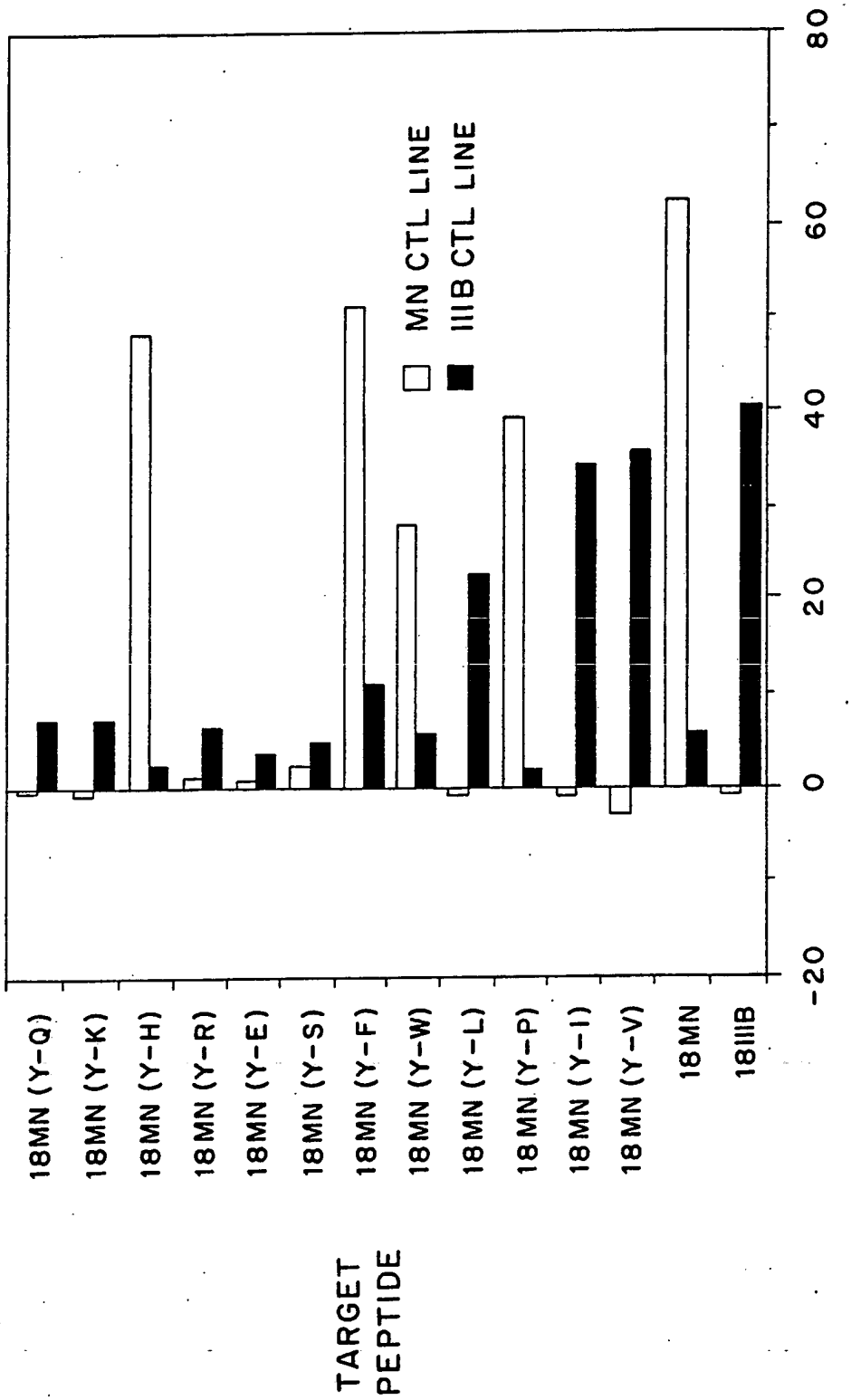
80
60
40
20
0
-20
% SPECIFIC LYSIS
MN CTL LINE
IIIB CTL LINE
18MN (Y-Q)
18MN (Y-K)
18MN (Y-H)
18MN (Y-R)
18MN (Y-E)
18MN (Y-S)
18MN (Y-F)
18MN (Y-W)
18MN (Y-L)
18MN (Y-P)
18MN (Y-I)
18MN (Y-V)
18MN
18IIIB
TARGET PEPTIDE

FIG.1

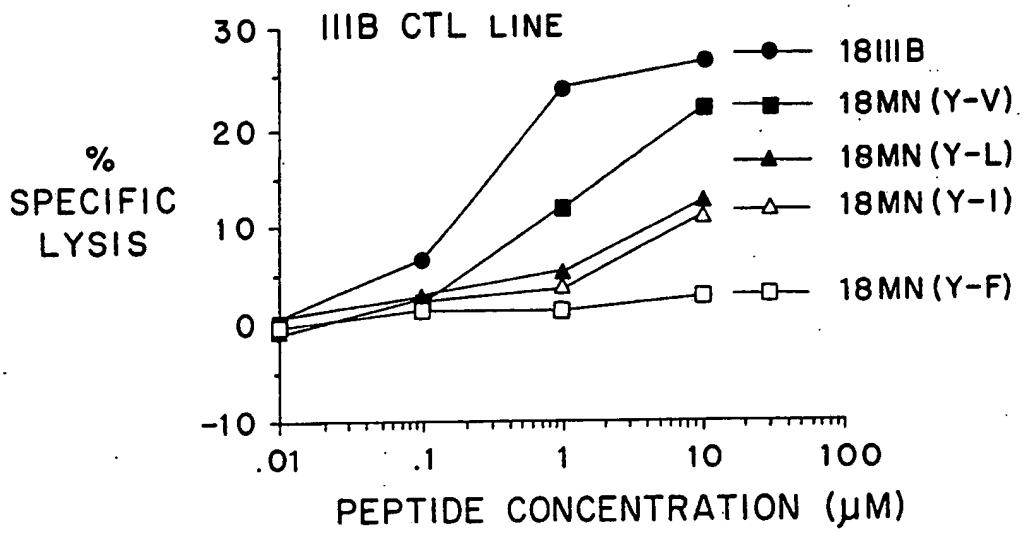
IIIB CTL LINE
18IIIB
18MN (Y-V)
18MN (Y-L)
18MN (Y-I)
18MN (Y-F)
% SPECIFIC LYSIS
30
20
10
0
-10
.01
.1
1
10
100
PEPTIDE CONCENTRATION (μM)

FIG. 2A

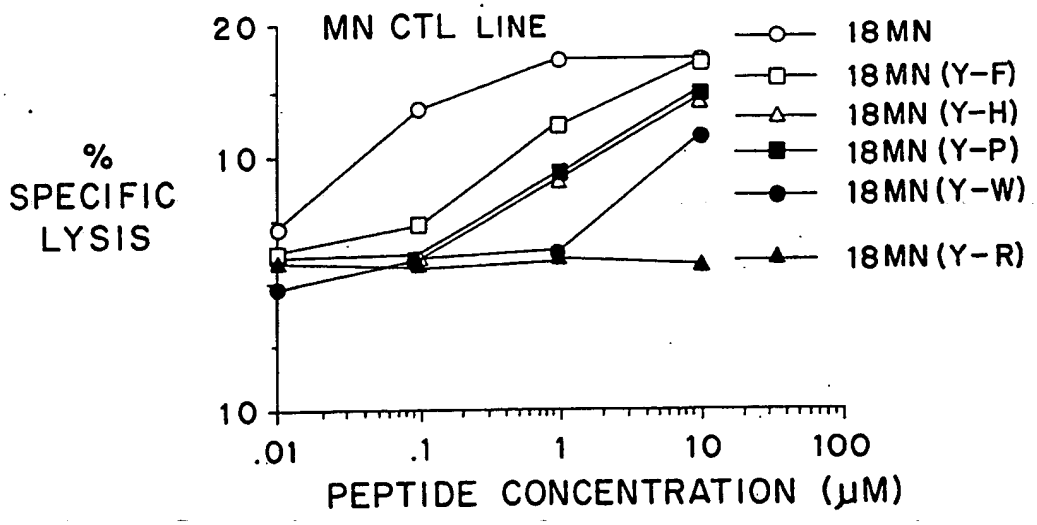
MN CTL LINE
18 MN
18 MN (Y-F)
18MN (Y-H)
18MN (Y-P)
18MN (Y-W)
18MN (Y-R)
% SPECIFIC LYSIS
20
10
10
.01
.1
1
10
100
PEPTIDE CONCENTRATION (μM)

FIG. 2B

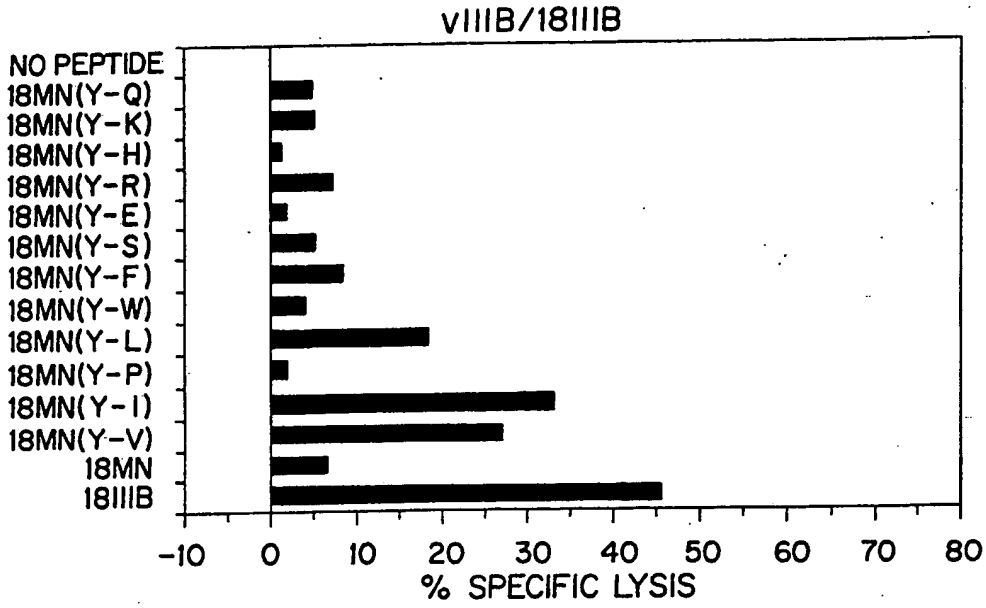
VIIIB/18IIIB
NO PEPTIDE
18MN(Y-Q)
18MN(Y-K)
18MN(Y-H)
18MN(Y-R)
18MN(Y-E)
18MN(Y-S)
18MN(Y-F)
18MN(Y-W)
18MN(Y-L)
18MN(Y-P)
18MN(Y-I)
18MN(Y-V)
18MN
18IIIB
-10 0 10 20 30 40 50 60 70 80
% SPECIFIC LYSIS

FIG. 3A

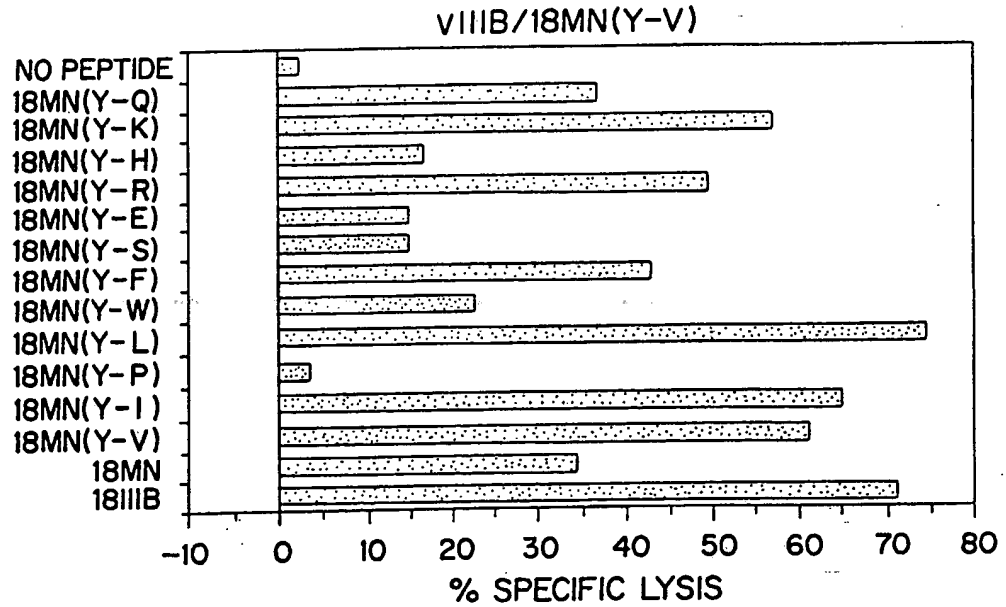
VIIIB/18MN(Y-V)
NO PEPTIDE
18MN(Y-Q)
18MN(Y-K)
18MN(Y-H)
18MN(Y-R)
18MN(Y-E)
18MN(Y-S)
18MN(Y-F)
18MN(Y-W)
18MN(Y-L)
18MN(Y-P)
18MN(Y-I)
18MN(Y-V)
18MN
18IIIB
-10 0 10 20 30 40 50 60 70 80
% SPECIFIC LYSIS

FIG. 3B

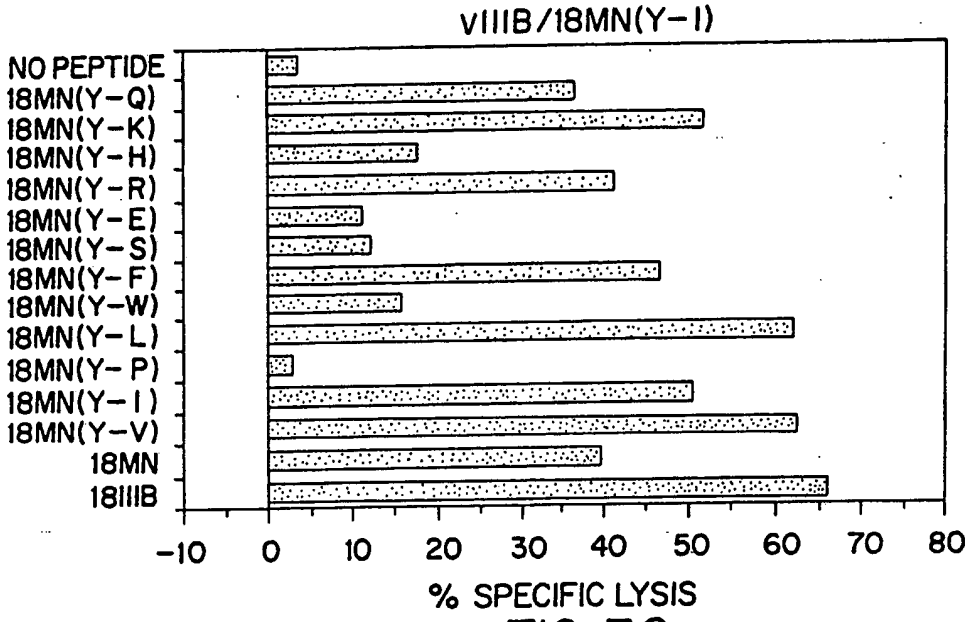
VIIIB/18MN(Y-I)
NO PEPTIDE
18MN(Y-Q)
18MN(Y-K)
18MN(Y-H)
18MN(Y-R)
18MN(Y-E)
18MN(Y-S)
18MN(Y-F)
18MN(Y-W)
18MN(Y-L)
18MN(Y-P)
18MN(Y-I)
18MN(Y-V)
18MN
18IIIB
-10 0 10 20 30 40 50 60 70 80
% SPECIFIC LYSIS

FIG. 3C

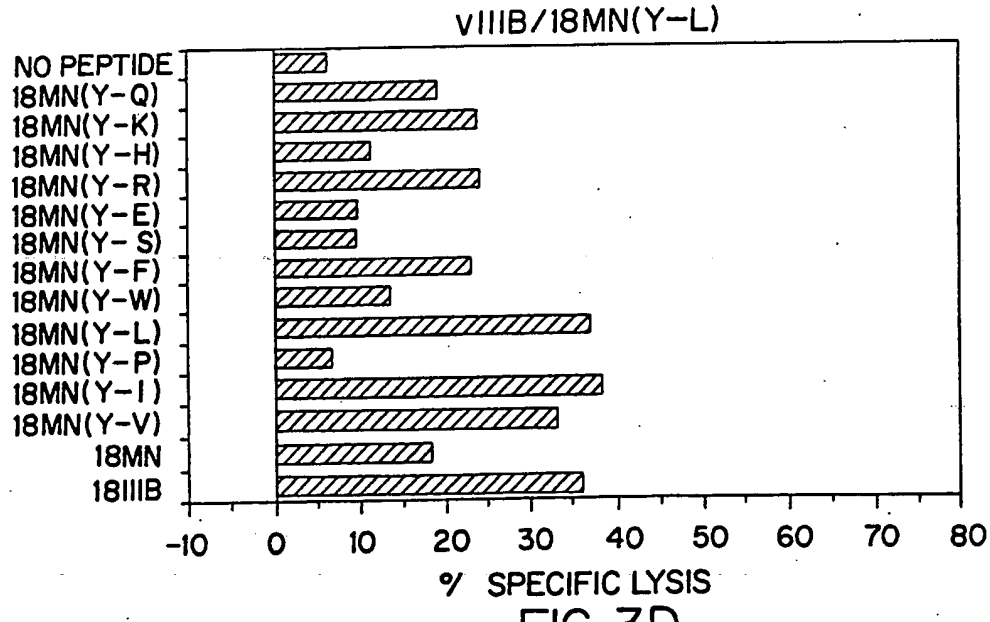
VIIIB/18MN(Y-L)
NO PEPTIDE
18MN(Y-Q)
18MN(Y-K)
18MN(Y-H)
18MN(Y-R)
18MN(Y-E)
18MN(Y-S)
18MN(Y-F)
18MN(Y-W)
18MN(Y-L)
18MN(Y-P)
18MN(Y-I)
18MN(Y-V)
18MN
18IIIB
-10 0 10 20 30 40 50 60 70 80
% SPECIFIC LYSIS

FIG. 3D

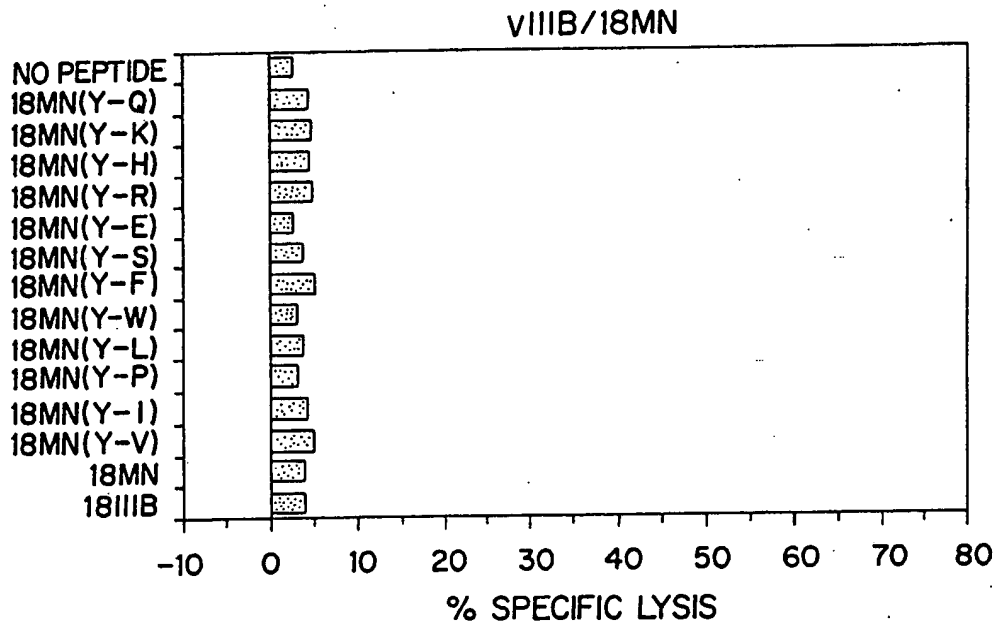
VIIIB/18MN
NO PEPTIDE
18MN(Y-Q)
18MN(Y-K)
18MN(Y-H)
18MN(Y-R)
18MN(Y-E)
18MN(Y-S)
18MN(Y-F)
18MN(Y-W)
18MN(Y-L)
18MN(Y-P)
18MN(Y-I)
18MN(Y-V)
18MN
18IIIB
-10 0 10 20 30 40 50 60 70 80
% SPECIFIC LYSIS

FIG. 3E

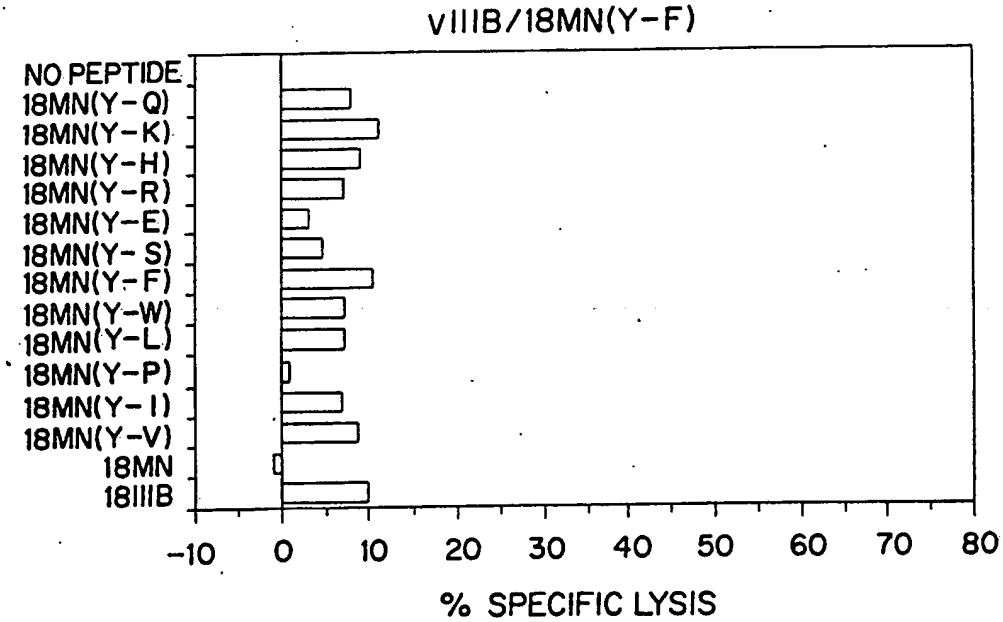
VIIIB/18MN(Y-F)
NO PEPTIDE
18MN(Y-Q)
18MN(Y-K)
18MN(Y-H)
18MN(Y-R)
18MN(Y-E)
18MN(Y-S)
18MN(Y-F)
18MN(Y-W)
18MN(Y-L)
18MN(Y-P)
18MN(Y-I)
18MN(Y-V)
18MN
18IIIB
-10 0 10 20 30 40 50 60 70 80
% SPECIFIC LYSIS

FIG. 3F

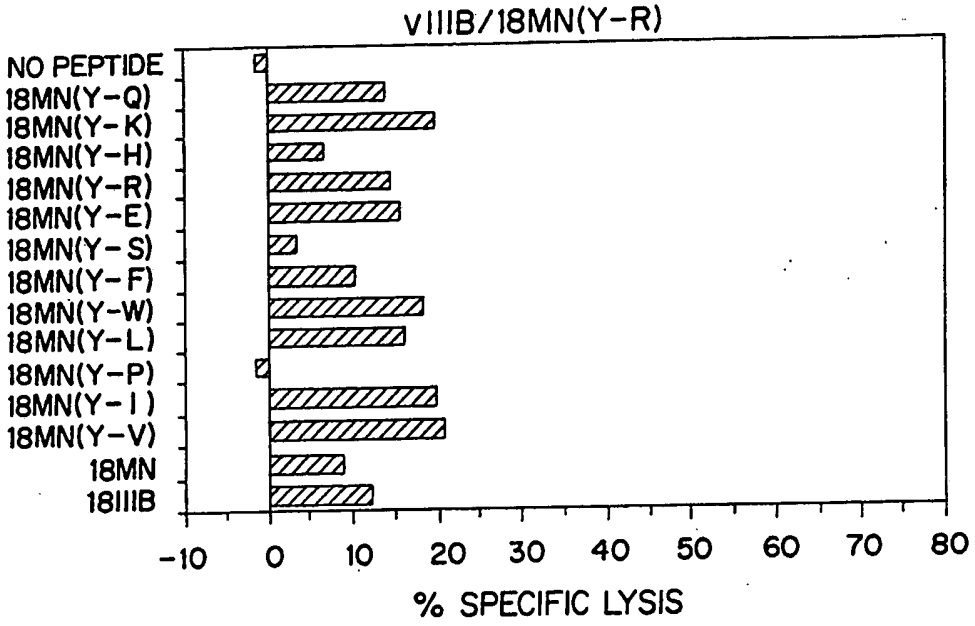
VIIIB/18MN(Y-R)
NO PEPTIDE
18MN(Y-Q)
18MN(Y-K)
18MN(Y-H)
18MN(Y-R)
18MN(Y-E)
18MN(Y-S)
18MN(Y-F)
18MN(Y-W)
18MN(Y-L)
18MN(Y-P)
18MN(Y-I)
18MN(Y-V)
18MN
18IIIB
-10 0 10 20 30 40 50 60 70 80
% SPECIFIC LYSIS

FIG. 3G

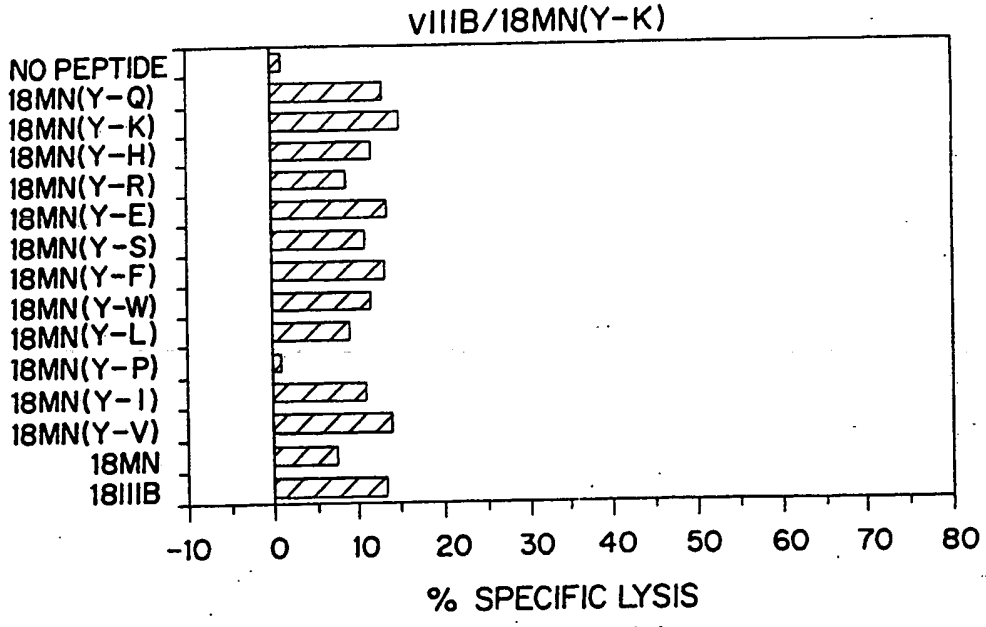
VIIIB/18MN(Y-K)
NO PEPTIDE
18MN(Y-Q)
18MN(Y-K)
18MN(Y-H)
18MN(Y-R)
18MN(Y-E)
18MN(Y-S)
18MN(Y-F)
18MN(Y-W)
18MN(Y-L)
18MN(Y-P)
18MN(Y-I)
18MN(Y-V)
18MN
18IIIB
-10 0 10 20 30 40 50 60 70 80
% SPECIFIC LYSIS

FIG. 3H

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/07714

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/21; C07K 7/08
US CL :530/326; 424/89
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/326; 424/89; 435/172.3, 235.1; 935/65; 930/224

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOGUE, REGISTRY
SEARCH TERMS: HIV, HTLV, V3, VACCIN?, IMMUNIZ?, SUBSTITUTION, ENVELOPE, HYPERVARIABLE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AIDS RESEARCH AND HUMAN RETROVIRUSES, VOLUME 6, NUMBER 10, ISSUED OCTOBER 1990, A. ROBERT NEURATH ET AL, "SEARCH FOR EPITOPE-SPECIFIC ANTIBODY RESPONSES TO THE HUMAN IMMUNODEFICIENCY VIRUS (HIV-1) ENVELOPE GLYCOPROTEINS SIGNIFYING RESISTANCE TO DISEASE DEVELOPMENT", PAGES 1183-1192.	1-19
A	AIDS RESEARCH AND HUMAN RETROVIRUSES, VOLUME 6, NUMBER 7, ISSUED JULY 1990, NANCY L. HAIGWOOD ET AL, "IMPORTANCE OF HYPERVARIABLE REGIONS OF HIV-1 GP120 IN THE GENERATION OF VIRUS NEUTRALIZING ANTIBODIES", PAGES 855-869.	1-19
Y	SCIENCE, VOLUME 246, ISSUED 06 OCTOBER 1989, HIDEMI TAKAHASHI ET AL, "A SINGLE AMINO ACID INTERCHANGE YIELDS RECIPROCAL CTL SPECIFICITIES FOR HIV-1 GP160", PAGES 118-121, ENTIRE DOCUMENT.	1-19
A	HUMAN RETROVIRUSES, ISSUED 1990, GOUDSMIT ET AL, "GENOMIC AND ANTIGENIC VARIATION IN THE GP120 NEUTRALIZATION DOMAIN V3: CHALLENGE TO AN AIDS VACCINE." PAGES 283-292, ENTIRE DOCUMENT.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 November 1992	Date of mailing of the international search report 04 DEC 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer CHRIS DUBRULE Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*